AD _____

Award Number: W81XWH-07-1-0303

TITLE: COX-1 Suppression and Follicle Depletion in the Etiology of
(Menopause-Associated Ovarian Cancer

PRINCIPAL INVESTIGATOR: Martin G. Belinsky, Ph.D.
(

CONTRACTING ORGANIZATION:
(
                    Fox Chase Cancer Center
                    333 Cottman Avenue, Philadelphia, PA 19111

REPORT DATE: October 2008
(

TYPE OF REPORT: Annual
(

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                 Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)
                 ✓ Approved for public release; distribution unlimited

                   Distribution limited to U.S. Government agencies only;
                   report contains proprietary information

The views, opinions and/or findings contained in this report are those of the author(s) and
should not be construed as an official Department of the Army position, policy or decision
unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3 DATES COVERED (From - To)
31-10-2008	Annual	01 APR 2007 - 30 SEP 2008

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
COX-1 Suppression and Follicle Depletion in the Etiology of Menopause-Associated Ovarian Cancer	5b. GRANT NUMBER — W81XWH-07-1-0303
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
Martin G. Belinsky, Ph.D.	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT
Fox Chase Cancer Center 333 Cottman Avenue Philadelphia, Pennsylvania 19111 E-Mail: Martin.Belinsky@fccc.edu	

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

✓ Approved for public release; distribution unlimited
  Distribution limited to U.S. Government agencies only - report contains proprietary information

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Menopause is defined as a permanent cessation of menstruation resulting from depletion of germs cells and loss of ovarian follicular activity. Menopausal ovaries undergo morphological changes that are likely related to the increased incidence of ovarian cancer in the peri- and post-menopausal periods. The germ cell-deficient Wv mice recapitulate these post-menopausal alterations in ovarian morphology and develop tubular adenomas. Genetic deletion of cyclooxygenase 1 (COX-1) delays germ cell depletion and preserves ovarian follicles and substantially delays the tumor development. Pharmacological inhibitors of COX-1 also rescued the tumor phenotype and preserved primary follicles in aged mice. These findings suggest that COX-1 activity may contribute to preneoplastic morphological changes of the ovarian surface epithelium, which can potentially be prevented by pharmacological inhibitors of COX-1. Moreover, the observations indicate that depletion of follicles may underlie the etiological factors that influence ovarian cancer risk.

15. SUBJECT TERMS

Ovary, cyclooxygenase-1, tumor, follicle

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Martin G. Belinsky, Ph.D.
a. REPORT	b. ABSTRACT	c. THIS PAGE		12	19b. TELEPHONE NUMBER
Unclassified	Unlimited	Unclassified			215-728-2756

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Martin G. Belinsky, Ph.D.

Table of Contents

Introduction

The research funded by this Ovarian Concept Award explores the hypothesis that the gonadotropin-stimulated inflammation-like reaction that occurs during the peri- and immediate post-menopause periods causes remodeling and morphological changes that promote ovarian cancer by selecting tumor-prone cells. COX-1 is most likely involved in the degeneration of ovarian follicles, and inhibition of COX-1 may reduce the gonadotropin levels and preserve ovarian function. Preservation of follicles may delay menopause and reduce the risk of ovarian cancer. Three specific aims were proposed: *Aim 1*) Genetic analysis to determine if COX-1 deletion alters tumor formation and the rate of follicle degeneration; *Aim 2*) Targeted COX inhibition to determine whether COX-1 is selective for follicle atresia and tumor phenotype; and *Aim 3*) Evaluation of COX-1 depletion/inhibition on ovarian physiology.

The experiments have established and studied a mouse model that incorporates postmenopausal biology and ovarian function in establishing ovarian cancer etiology and risk. Three mouse lines were established and/or maintained for these experiments: 1) Wv mutant mice (C57BL/6J-Kit$^{W-v/J}$ from Jackson Laboratory) contain a point mutation in the kinase domain of c-Kit, are viable, and lack pigment-forming cells, germ cells, red blood cells, and mast cells. The females are sterile, and their ovaries have an aged, menopause-like phenotype. 2) COX-1 homozygous mutant mice, from Langenbach and colleagues (NIEHS, Research Triangle Park, NC), develop normally, have no gastric pathology, are fertile, but have been reported to have parturition defects. 3) Wv were intercrossed with COX-1 mutant mice to generate Wv/Wv:Cox-1(-/-).

Body

We examined the naturally occurring mutant mouse, the white spotting variant (Wv) mice to understand the relationship between the development of age-related morphological changes in the ovary and depletion of ovarian oocytes and follicles. These mice harbor a point mutation in the kinase domain of the c-kit gene, resulting in developmental defects in germ cells, pigment-forming cells, red blood cells, and mast cells in homozygous mutant mice (1-3). At birth Wv homozygous mutant ovaries contain less that 1% of the normal number of oocytes, and the remaining oocytes are depleted by about 6-8 weeks of age (4). Consequently, ovulation ceases and levels of serum pituitary gonadotropins increase. Pronounced epithelial morphological changes develop, including surface invaginations, inclusion cysts, papillomatosis, and benign ovarian tumors, known as tubular adenomas that completely infiltrate the ovary by four months of age (3,5). The tumors are derived from ovarian surface epithelial cells, resembling human ovarian changes that may result from aging (5). Gonadotropins are believed to be the cause of these tubular adenomas, since suppression of gonadotropin release in Wv mice prevents the development of the ovarian tubular adenomas (6). The Wv mice over-express cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) (5), which are distinct enzymes expressed either constitutively or induced, respectively. The COX enzymes regulate multiple aspects of female reproduction (7), and non-steroidal anti-inflammatory drugs (NSAIDs) that target the COX enzymes reduce the risk of ovarian epithelial cancers, by far the most predominant form (8), and cause growth inhibition and apoptosis in ovarian cancer cell lines (9). However, the link between morphological inhibition and reproductive inhibition is unclear.

In **Aim 1**, we used a genetic approach to determine if COX-1 deletion alters tumor formation and the rate of follicle degeneration. Cox-1 deficiency was introduced into the Wv mouse colony by crossing Wv/+ mice with Cox-1(+/-) mice, and a new inbred colony was established by crossing Wv/+,Cox-1(+/-) siblings. Progenies homozygous for Wv and all genotypes of Cox-1 [(+/+), (+/-), (-/-)] were examined for ovarian morphology at 4 months. In wildtype mice, the ovaries contained follicles of all stages of development, as well as numerous corpora lutea, which develop from the Graafian follicles after ovulation. The presence of corpora lutea indicates that the wildtype ovaries have ovulated, and are reproductively competent **(Fig. 1)**.
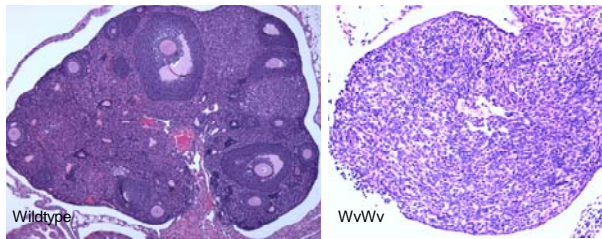

Fig. 1. Age-matched wildtype (left panel) and Wv/Wv (right panel) ovaries. Follicles of all stages and sizes are found in four-month old wildtype mouse ovaries. The Wv/Wv ovary lacks detectable follicles.

The Wv mouse ovaries were smaller, void of detectable follicles at any stage, and infiltrated throughout with cytokeratin- positive lesions known as tubular adenomas. No obvious follicles can be detected in the Wv ovaries. Genetic deletion of COX-1 was dosage dependent, with the homozygous mutant having the most pronounced effect on the ovarian morphology, with few or negligible lesions **(Fig. 2)**. The COX-1 deletion in the Wv/Wv background reduced the area infiltrated by the lesions nearly 3-fold. A double mutant, Wv/Wv,Cox-1(+/-),Cox-2(+/-), was as effective as the COX-1 homozygous in reducing the tumor phenotype.
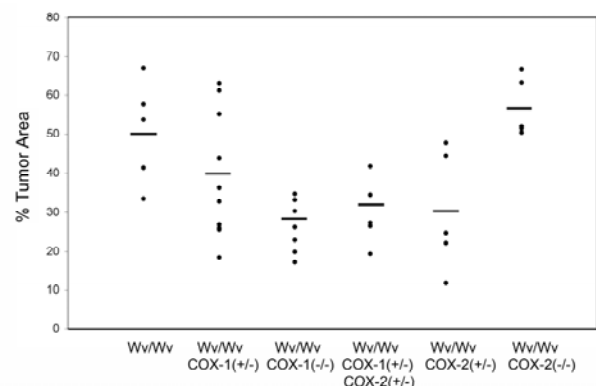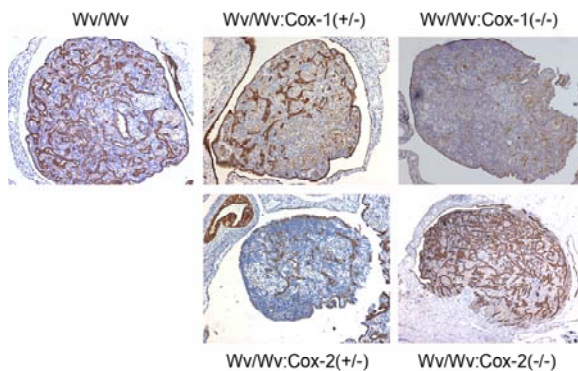

Fig. 2. **(Left panel)** Troma-1 (Cytokeratin-8) staining of 4-month old ovaries from mice homozygous for Wv and all genotypes of Cox-1
(+/+, +/-, -/-) shows the infiltration of epithelial tubular adenomas within the ovaries. The most significant reduction in tumor infiltration was found in the Wv/Wv, Cox-1(-/-) ovaries. **(Right panel)** The percent tumor area was determined for individual ovaries of the defined genotypes and plotted. Ovaries from Wv/Wv mice with the heterozygous Cox-1,Cox-2 genotype [Cox-1(+/-),Cox-2(+/-)] and Wv/Wv, Cox-2(+/-) genotype also show a rescue of the tumor phenotype.

Just prior to the initiation of this award, we had found that deletion of Cox-2 in the Wv background resulted in an increase in the expression of Cox-1 in the Wv/Wv,Cox-2(-/-) ovaries, which was not found in the Wv/Wv,Cox-2(+/-) ovaries (1). In our current studies, we have not found a corresponding change in Cox-2 levels in the Wv/Wv,Cox-1(-/-) ovaries (Fig. 3). Collectively, this data supports the hypothesis that Cox-1 expression is pertinent to the development of the tumors in the Wv/Wv mice.
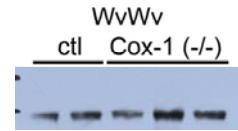

Fig. 3. Cox-2 expression does not change in Wv/Wv,Cox-1 knockout mice.

In 1.5-month old mice, COX-1 deletion appears to slow the loss of follicles retained in the Wv/Wv ovary. For example, we have preliminarily observed widespread immunostaining of inhibin-alpha, a granulosa cell marker, and PGC-7, a germ cell marker, in Wv/Wv,Cox-1(-/-) mice, which would indicate that COX-1 inhibition retains the oocytes and follicular structure. A reasonable question, then, is whether the Cox-1 genetic knockout also increases the number of primordial or primary follicles found in young mice. We have begun to examine newborn ovaries for germ cells and primordial follicles and expect to determine if COX-1 genetic inhibition delays the depletion of the follicles and at what step of follicle development.

In **Aim 2**, pharmacological cyclooxygenase inhibitors were administered to Wv/Wv mice to analyze whether COX-1 is selective for the tumor phenotype and follicle atresia. Female Wv/Wv mice were treated with inhibitors of COX isoforms to determine that COX-1 is the principal COX involved in ovarian morphological changes. The drugs were administered in food or drinking water for 2 months, and ovaries were collected from mice at 4 months of age. We had reported previously that Celebrex, a COX-2 specific inhibitor, could suppress the Wv/Wv ovarian tumor phenotype (1). Here, we found that indomethacin, a non-selective COX inhibitor, and SC-560, a COX-1 specific inhibitor, were much more effective. In animals treated with either of these two drugs, the tumor phenotype was reduced approximately 80% (from 80% to 17% tumor area). Moreover, in 11 out of 13 mice treated with COX-1 inhibitors, one or more follicles were retained in the ovary **(Fig. 4)**.
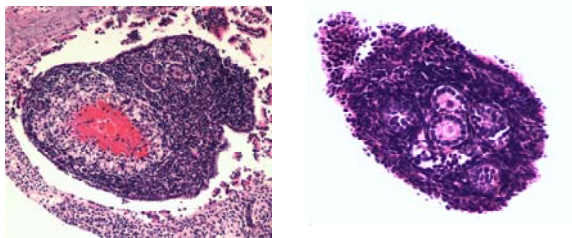

Fig. 4. Ovaries from 4-month old Wv/Wv mice treated with Cox-1 inhibitors for 2 months show a rescue of the tumor phenotype and the presence of pre-antral follicles, which are not found in non-treated Wv/Wv mice of the same age.

Based on the presence of a single layer of granulosa cells surrounding the oocyte and the size of the oocyte, the follicles appeared to be stage III primary follicles (3). The presence of a follicle in the ovary of a 4-month old Wv/Wv female was quite remarkable, since in over 300 ovaries examined in the course of our studies with the Wv mice we have never observed a follicle in mature Wv/Wv ovaries.

Finally, in **Aim 3**, we proposed to evaluate the effect of COX-1 depletion or inhibition on the ovarian phenotype. Our preliminary measurements showed that deletion of COX-1 lowered serum FSH levels, though the results were not significant **(Fig. 5)**. A larger sample number is required to make a solid conclusion. We have begun to validate a commercially available EIA (from ALPCO Diagnostics) to replace the RIA that we had outsourced to the National Hormone and Peptide Program (Harbor-UCLA Research and Education Institute). Other measurements, including prostaglandins, progesterone, and estrogen, were initially proposed and will be followed up in the future.
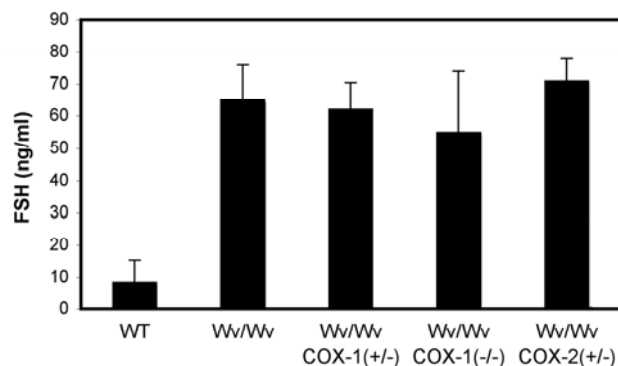

Fig. 5. Serum FSH levels in 4 month Wv/Wv mice. FSH was measured in serum obtained by retro-orbital bleed at the time of sacrifice from Wv/Wv mice of different Cox-1 genotypes, and determined by radioimmunoassay through custom service by the National Hormone and Peptide Program (Harbor-UCLA Medical Center, Torrance, CA).

Our request to transfer the Principal Investigator from Elizabeth Smith, Ph.D. to Martin G. Belinsky, Ph.D. and a revised statement of work was approved during this last report period.

Key Research Accomplishments

- Found that COX-1 genetic depletion rescues the ovarian epithelial tumor phenotype of the Wv mice.
- Determined that the effect of COX-1 knockout is gene dosage-dependent, with the homozygous mutant having the greatest effect on ovarian morphology.
- Determined that COX-2 expression does not compensate for COX-1 in Wv/Wv,COX-1(-/-) mice.
- Assessed that COX-1 deletion delays the depletion of follicles in the younger mice and consequently the development of the epithelial lesions in the Wv mice.
- Showed that pharmacological inhibition of COX-1 was effective in rescuing the tumor phenotype and more effective than COX-2 inhibitors.
- Found that COX-1 inhibition appears to influence the preservation of primary follicles.

Reportable Outcomes

One manuscript has been prepared during the first year of the award.

Smith ER, and Xu XX. Ovarian aging, follicle depletion, and cancer: a hypothesis for the etiology of epithelial ovarian cancer involving follicle depletion. *Lancet Oncol.* 2008, accepted.

Conclusions

These studies have examined the hypothesis that gonadotropins stimulate an inflammatory-like reaction, mediated through cyclooxygenase-1 action, which causes remodeling and morphological changes and promotes ovarian tumor development. We have utilized a practical model for post-menopausal ovarian physiology, the Wv mouse, in which cyclooxygenase-1 is over-expressed, as it is frequently found in ovarian epithelial cancers. Genetic and pharmacological inhibition of COX-1 prevented the tumor phenotype and delayed the depletion of follicles. Importantly, the depletion of follicles may underlie the epidemiological observations linking reproductive history and ovarian cancer risk. The use of non-steroidal anti-inflammatory agents that target cyclooxygenases may provide a rational strategy for chemoprevention of ovarian cancer.

References

1. Reith AD, Rottapel R, Giddens E, Brady C, Forrester L, Berstein A. W mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-kit receptor. *Genes Dev* 1990;**4**:390-400.
2. Mintz B. Embryological development of primordial germ-cells in the mouse: influence of a new mutation, Wj. *J Embryol Exp Morphol* 1957;**5**:396-406.
3. Murphy ED, Beamer WG. Plasma gonadotropin levels during early stages of ovarian tumorigenesis in mice of the Wx-Wv genotype. *Cancer Res* 1973;**33**:721-3.
4. Murphy ED. Hyperplastic and early neoplastic changes in the ovaries of mice after genic deletion of germ cells. *J Natl Cancer Inst* 1972;**48**:1283-95.
5. Yang WL, Cai KQ, Smith ER, Klein-Szanto A, Hamilton TC, Xu XX. A reduction of cyclooxygenase 2 gene dosage counters the ovarian morphological aging and tumor phenotype in Wv mice. *Am J Pathol* 2007;**170**:1325-36.
6. Blaaker J, Baeksted M, Micic S, Albrectsen P, Rygaard K, Bock J. Gonadotropin-releasing hormone agonist suppression of ovarian tumorigenesis in mice of the Wx/Wv genotype. *Biol Reprod* 1995;**53**:775-9.
7. Langenbach R, Loftin C, Lee C, Tiano H. Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. *Biochem Pharmacol* 1999;**58**:1237-46.
8. Barnes MN, Grizzle WE, Grubbs CJ, Partridge EE. Paradigms for primary prevention of ovarian Carcinomas. *CA Cancer J Clin* 2002;**52**:216-25.
9. Rodriguez-Burford C, Barnes MN, Oelschlager DK, Myers RB, Talley LI, Partridge EE, and Grizzle WE. Effects of nonsteroidal anti-inflammatory agents (NSAIDs) on ovarian carcinoma cell lines: preclinical evaluations of NSAIDs as chemopreventive agents. *Clin Cancer Res* 2002;**8**:202-9.

Appendices

Smith ER, and Xu XX. Ovarian aging, follicle depletion, and cancer: a hypothesis for the etiology of epithelial ovarian cancer involving follicle depletion. *Lancet Oncol.* 2008, accepted. (Editorial draft is provided.)

Ovarian ageing, follicle depletion, and cancer: an hypothesis for the aetiology of epithelial ovarian cancer involving follicle depletion

Elizabeth R Smith, Xiang-Xi Xu [A1]

The association between ovarian-cancer risk and reproductive factors has been well established, and two main theories, incessant ovulation and gonadotropin stimulation, have been proposed to explain the mechanism. Recent studies using animal models of ovarian tumorigenesis, and analysis of ovarian tissues from prophylactic oophorectomies, suggest that depletion of ovarian follicles might underlie the epidemiological findings linking reproductive history and ovarian-cancer risk.

Introduction [A13]

The aetiology of ovarian cancer is complex and incompletely understood, although epidemiological data decisively link ovulation frequency and reproductive hormones to ovarian-cancer risk.[1–3] Increased parity and oral contraceptive use are the clearest examples of factors that decrease ovarian-cancer risk, both of which limit ovulation. Two main theories, incessant ovulation[4] and gonadotropin stimulation,[5,6] have been proposed to explain the aetiology of ovulation in ovarian-cancer risk,[7,8] but neither completely nor satisfactorily explain the dramatic increase in ovarian-cancer incidence that occurs in the immediate postmenopausal period and that continues to rise as the ovary ages after menopause. Most (90%) ovarian cancers are derived from the surface epithelium, and nearly 85–90% of ovarian cancer develops postmenopause. Therefore, understanding how the depletion of germ cells, loss of the follicular structure that surrounds the oocyte or germ cell, and cessation of ovulation, all of which define menopause, effect transformation of the surface epithelium and the development of ovarian carcinomas is of particular importance [A3]. On the basis of studies with the germ-cell-deficient Wv mice (figure 1) and examination of

human ovarian tissues from prophylactic oophorectomies, we suggest that depletion of germ cells and the loss of ovarian follicular function that follows might underlie the link between reproductive factors and ovarian cancer risk.

Aetiology of ovulation in ovarian cancer

Nearly 40 years ago, Fathalla proposed the theory of incessant ovulation to clarify the association between ovulation frequency and the risk of developing epithelial ovarian cancer.[4] This hypothesis attributes the occurrence of ovarian cancer in modern day women (and domestic egg-laying hens), which is rare in other mammals, to ovulation that recurs monthly throughout the reproductive lifetime of women if not punctuated by anovulatory periods during pregnancy and breast-feeding.[4] The repetitive wounding during the release of the ovum and the cell proliferation that occurs postovulation to repair the ovarian surface epithelium have been proposed to result in mutations accumulating in the epithelial cells and ultimately the formation of tumours.[9] This central mechanism is supported experimentally in cell culture and is generally well accepted. Moreover, combined oestrogen–progesterone formulations of oral contra-

Department of Medicine
(E R Smith PhD)**, Sylvester Comprehensive Cancer Center**
(E R Smith, X-X Xu)**, and Department of Obstetrics and Gynecology** (X-X Xu)**, University of Miami School of Medicine, Miami, FL, USA**

Correspondence to:
Dr Elizabeth R Smith, [A2]
University of Miami School of Medicine, Miami, FL 33136, USA
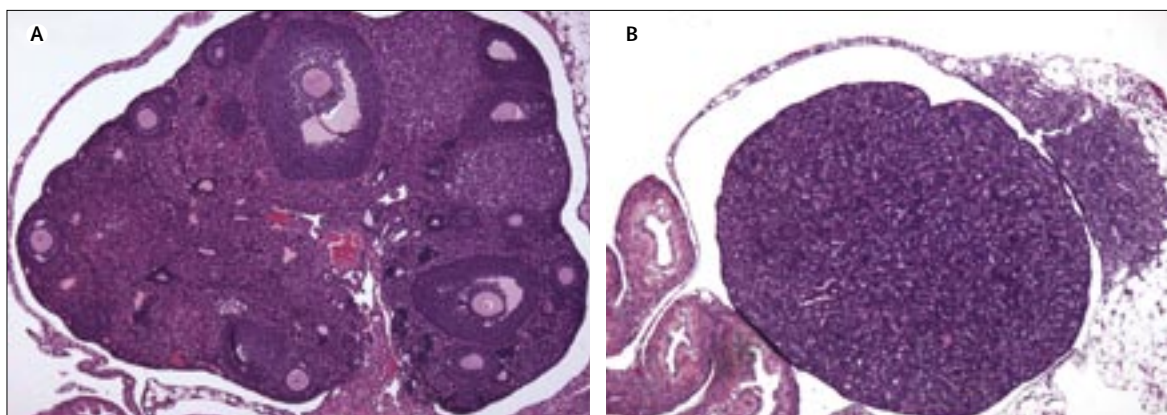**esmith@med.miami.edu**

Figure 1: [A10]
Mature wildtype-mouse ovary contains many germ cells and follicles at various stages of development, including primary, preantral, antral, and corpora lutea. A Wv ovary from the same-age mouse is depleted of germ cells and follicles and many epithelial morphological changes, including tubular adenomas, permeate the ovary [A11].
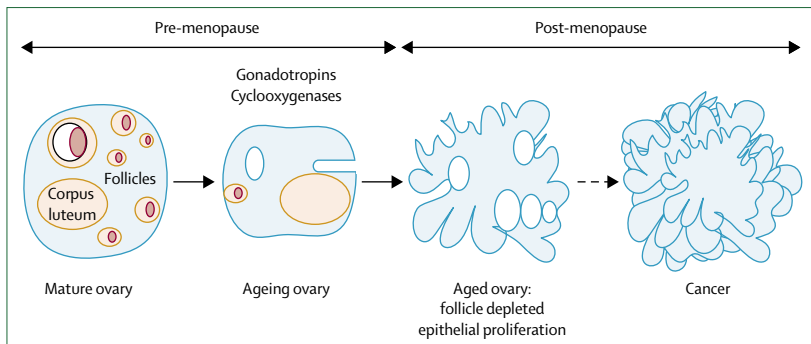
Figure 2: Model depicting hypothesis that germ-cell and follicle depletion might underlie the aetiology of ovarian-cancer risk associated with reproductive factors and menopause risk

ceptives that act by suppressing ovulation decrease the risk of ovarian cancer by about 40% after 3 years of use.[10,11] Why pregnancy and oral contraceptives provide a long-term protection, however, might be more complex than by simply limiting ovulation, because more recent studies suggest that progesterone, which is increased during pregnancy and by oral contraceptives, might also affect the clearing of transformed cells from the ovarian surface-epithelial layers.[12,13] Because the likelihood of initiated cells [A4] being present increases with age, the age of either the last full-term pregnancy or the last regular use of progestin-containing oral contraceptives is a protective factor, and the benefit decreases after time.[13]

[A14] On the basis of the same epidemiological data, the gonadotropin stimulation theory, by contrast, postulates that surges of pituitary gonadotropins that initiate each ovulation and persist in high levels for many years after menopause also stimulate the ovarian surface-epithelial cells and induce cell transformation.[5,6] During ovulation, gonadotropins stimulate an inflammatory-like process mediated by many cytokines and proteolytic enzymes, which leads to rupture of the ovarian surface-epithelial layer for the release of the ovum.[14–16] After rupture of the follicle and the release of the ovum at the ovarian surface, the oestrogen-producing follicle is converted to a progesterone-producing corpus luteum, which then feeds back to inhibit gonadotropin levels. In menopause, which is caused by a total depletion in the number of germ cells present in the ovary and accompanied by the loss of the follicular structure that surrounds the germ cells, the feedback endocrine loop from the corpus luteum is absent, and serum gonadotropins and levels of proinflammatory cytokines are even higher in perimenopausal ovaries.[17,18] Thus, increased gonadotropin levels in postmenopausal women might foster an inflammatory environment that cannot lead to ovulation, but might contribute to ovarian-cancer risk,[19] by causing either remodeling or morphological changes in the surface epithelium, which allow the transformation of genetically compromised cells and the development of cancerous lesions.[20,21] This theory seems more fitting to explain the dramatic increase in ovarian-

cancer incidence in the perimenopausal and postmenopausal years.[22–24] The average age of menopause, although it varies somewhat between women and cultures, is 51 years, which closely precedes the average age of ovarian-cancer diagnosis, which is 54 years.

[A15] The incidence of ovarian cancer continues to increase after menopause, and age—even more so than a family history of ovarian cancer—is the best prediction of ovarian-cancer risk.[1–3] Ovaries from older women have been noted to show more morphological changes than those from younger women.[25] Presumably ovulation and subsequent repair cause these age-dependent changes, or so-called ovarian ageing,[23,26] which might represent preneoplastic areas or lesions. Recently, prophylactic surgeries have allowed a closer and crucial examination of these morphological changes and potential preneoplastic lesions in healthy women.[27–29] These morphological features include papillomatosis, deep-surface invaginations, inclusion cysts, and epithelial stratification/proliferation [A5], which are noted most frequently in perimenopausal and immediate postmenopausal ovaries of both *BRCA1/2* carriers and non-carriers,[25] and might very well be the result of ovarian ageing.

[A16] Recent laboratory studies of the germ-cell-deficient Wv mouse also provide intriguing ideas about the aetiology of ovarian cancer.[30] The Wv mice harbour a point mutation in the c-Kit gene that greatly decreases the tyrosine-kinase activity of c-Kit, affecting the development of germ cells, pigment cells, and mast cells.[31] Homozygous Wv-mutant mice have a similar lifespan as wildtype mice, but are essentially sterile due to the germ-cell defect.[32] Wv/Wv females contain less than 1% of the normal number of oocytes at birth, and once reproductive age is reached, ovarian follicles are rapidly depleted.[33] Subsequently, serum gonadotropins are increased and substantial epithelial morphological changes develop, including surface invaginations, inclusion cysts, papillomatosis, and benign ovarian tumours, known as tubular adenomas.[30,34] These tumours are derived from ovarian surface-epithelial cells, resembling human ovarian changes that might result from ageing.[30] In the absence of germ cells, as in the Wv mouse, suppression of gonadotropin release prevents the development of the ovarian tubular adenomas,[35] yet gonadotropin administration alone (ie, germ cells are present in the ovaries) does not result in epithelial tumours.[36,37] Moreover, transgenic mouse models targeting the gonadotropin pathway without depletion of oocytes do not develop epithelial tumours. Female mice overexpressing follicle-stimulating hormone (FSH) in levels far exceeding those in postmenopausal women develop haemorrhagic and cystic ovaries, but do not develop epithelial lesions.[38] Additionally, genetic knockouts of the FSH receptor in the ovary, in which circulating serum FSH levels are high but non-functioning, do not develop epithelial cancers, but instead

sex-cord tumours, which represent only a small subset of human ovarian cancers.[39] Thus, the early depletion of germ cells and subsequent increase in gonadotropins in the Wv mice might constitute a relevant, albeit exaggerated model, mimicking the postmenopausal biology and ovarian morphological ageing in women.

Follicle depletion

Studies of the Wv mice prompted us to postulate that depletion of ovarian germ cells and follicles might underlie the aetiology of ovarian-cancer risk associated with reproductive factors and menopause status (figure 2), and might in fact unify incessant ovulation and gonadotropin stimulation as mechanisms.

Follicle depletion explains the age-dependent risk of ovarian cancer: ovarian cancer generally develops in the immediate postmenopausal years, when ovarian follicles are depleted. The depletion of ovarian follicles obviously precedes and causes increased serum gonadotropins, which stimulate an inflammatory environment in the ovary that is permissive to transformation of surface epithelial cells and tumour development. Furthermore, both incessant ovulation and gonadotropin stimulation will accelerate the depletion of ovarian follicles. Additionally, protective factors, such as the use of birth-control pills,[40] and cyclooxygenase inhibitors,[41–43] might preserve follicles. Cyclooxygenases participate in follicle development and ovulation,[44,45] and their inhibition might slow follicle maturation and extend follicle lifespan. Findings show that lifetime ovulation correlates with premenopausal, but not postmenopausal, ovarian-cancer risk,[46] and a decrease of pituitary gonadotropin release with hormone-replacement therapy does not decrease ovarian-cancer risk.[47] These findings also support the notion that the presence of ovarian follicles, rather than ovulation and gonadotropin stimulation, is a major determinant of ovarian-cancer risk.

Conclusion

The follicle-depletion hypothesis predicts that follicle preservation and delaying reproductive ageing might prevent ovarian cancer or decrease the risk of developing this disease, and that menopause timing might correlate with ovarian-cancer incidence, which can be verified by epidemiological studies designed to assess these factors.

Conflicts of interest
The authors declared no conflicts of interest.

> **Search strategy and selection criteria [A12]**
>
> Information for this Personal View was obtained by searches of ....[**Please list databases and other sources used, ie, PubMed, Medline, etc**], by use of the search terms .... Only papers published between .... and .... [**Please provide dates**] in ....[**please provide language**] were included.

References
1   Tortolero-Luna G, Mitchell MF. The epidemiology of ovarian cancer. *J Cell Biochem Suppl* 1995; **23:** 200–07.
2   Yancik R. Ovarian cancer. Age contrasts in incidence, histology, disease stage at diagnosis, and mortality. *Cancer* 1993; **71 (2 suppl):** 517–23.
3   Riman T, Persson I, Nilsson S. Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence. *Clin Endocrinol* 1998; **49:** 695–707.
4   Fathalla MF. Incessant ovulation—a factor in ovarian neoplasia? *Lancet* 1971; **2:** 163.
5   Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. *J Natl Cancer Inst* 1983; **71:** 717–21.
6   Mohle J, Whittemore A, Pike M, Darby S. Gonadotrophins and ovarian cancer risk. *J Natl Cancer Inst* 1985; **75:** 178–80.
7   Ozols RF, Bookman MA, Connolly DC, et al. Focus on epithelial ovarian cancer. *Cancer Cell* 2004; **5:** 19–24.
8   Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiol Biomarkers Prev* 2005; **14:** 98–107.
9   Godwin AK, Testa JR, Handel LM, et al. Spontaneous transformation of rat ovarian surface epithelial cells: association with cytogenetic changes and implications of repeated ovulation in the etiology of ovarian cancer. *J Natl Cancer Inst* 1992; **84:** 592–601.
10   Barnes MN, Grizzle WE, Grubbs CJ, Partridge EE. Paradigms for primary prevention of ovarian carcinomas. *CA Cancer J Clin* 2002; **52:** 216–25.
11   Hankinson SE, Colditz GA, Hunter DJ, Spencer TL, Rosner B, Stampfer MJ. A quantitative assessment of oral contraceptive use and risk of ovarian cancer. *Obstet Gynecol* 1992; **80:** 708–14.
12   Rodriguez GC, Walmer DK, Cline M, et al. Effect of progestin on the ovarian epithelium of macaques: cancer prevention through apoptosis? *J Soc Gynecol Investig* 1998; **5:** 271–76.
13   Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J Natl Cancer Inst* 1998; **90:** 1774–86.
14   Yang WL, Godwin AK, Xu XX. Tumor necrosis factor-alpha-induced matrix proteolytic enzyme production and basement membrane remodeling by human ovarian surface epithelial cells: molecular basis linking ovulation and cancer risk. *Cancer Res* 2004; **64:** 1534–40.
15   Talbot P, Martin GG, Ashby H. Formation of the rupture site in preovulatory hamster and mouse follicles: loss of the surface epithelium. *Gamete Res* 1987; **17:** 287–302.
16   Richards JS, Russell DL, Ochsner S, Espey LL. Ovulation: new dimensions and new regulators of the inflammatory-like response. *Annu Rev Physiol* 2002; **64:** 69–92.
17   Eldridge JC, McPherson JC 3rd, Mahesh VB. Maturation of the negative feedback control of gonadotropin secretion in the female rat. *Endocrinology* 1974; **94:** 1536–40.
18   Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proimflammatory cytokine activity after menopause. *Endocr Rev* 2002; **23:** 90–119.
19   Smith ER, Xu XX. Etiology of epithelial ovarian cancer: a cellular mechanism for the role of gonadotropins. *Gynecol Oncol* 2003; **91:** 1–2.
20   Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. *J Natl Cancer Inst* 1999; **91:** 1459–67. [A9]

Personal View

21 Smith ER, Daly MB, Xu XX. A mechanism for Cox-2 inhibitor anti-inflammatory activity in chemoprevention of epithelial cancers. *Cancer Epidemiol Biomarkers Prev* 2004; **13:** 144–45.

22 Gosden RG. Biology of menopause: the causes and consequences of ovarian aging. Burlington, MA: Academic Press, 1985.

23 Finn CA. Reproductive ageing and the menopause. *Int J Dev Biol* 2001; **45:** 613–17.

24 Lobo RA, Kelsey J, Marcus R, eds. Menopause: biology and pathobiology. 1st edn. Burlington, MA: Academic Press, 2000.

25 Cai KQ, Klein-Szanto A, Karthik D, et al. Age-dependent morphological alterations of human ovaries from populations with and without BRCA mutations. *Gynecol Oncol* 2006; **103:** 719–28.

26 Nicosia SV. The aging ovary. *Med Clin North Am* 1987; **71:** 1–9.

27 Salazar H, Godwin AK, Daly MB, et al. Microscopic benign and invasive malignant neoplasms and a cancer-prone phenotype in prophylactic oophorectomies. *J Natl Cancer Inst* 1996; **88:** 1810–20.

28 Barakat RR, Federici MG, Saigo PE, Robson ME, Offit K, Boyd J. Absence of premalignant histologic, molecular, or cell biologic alterations in prophylactic oophorectomy specimens from BRCA1 heterozygotes. *Cancer* 2000; **89:** 383–90.

29 Roland IR, Yang WL, Yang DH, et al. Loss of surface and cyst epithelial basement membranes and pre-neoplastic morphological changes in prophylactic oophorectomies. *Cancer* 2003; **98:** 2607–23.

30 Yang WL, Cai KQ, Smith ER, Klein-Szanto A, Hamilton TC, Xu XX. A reduction of Cox-2 gene dosage counters the menopausal ovarian morphological aging and tumor phenotype in Wv mice. *Am J Pathol* 2007; **170:** 1325–36.

31 Reith AD, Rottapel R, Giddens E, Brady C, Forrester L, Bernstein A. W mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-kit receptor. *Genes Dev* 1990; **4:** 390–400.

32 Mintz B. Embryological development of primordial germ-cells in the mouse: influence of a new mutation, Wj. *J Embryol Exp Morphol* 1957; **5:** 396–403.

33 Murphy ED. Hyperplastic and early neoplastic changes in the ovaries of mice after genic deletion of germ cells. *J Natl Cancer Inst* 1972; **48:** 1283–95.

34 Murphy ED, Beamer WG. Plasma gonadotropin levels during early stages of ovarian tumorigenesis in mice of the Wx-Wv genotype. *Cancer Res* 1973; **33:** 721–23.

35 Blaakaer J, Baeksted M, Micic S, Albrectsen P, Rygaard K, Bock J. Gonadotropin-releasing hormone agonist suppression of ovarian tumorigenesis in mice of the Wx/Wv genotype. *Biol Reprod* 1995; **53:** 775–79.

36 Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996; **383:** 531–35.

37 Vanderhyden BC. Loss of ovarian function and the risk of ovarian cancer. *Cell Tissue Res* 2005; **322:** 117–24.

38 Kumar TR, Palapattu G, Wang P, et al. Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. *Mol Endocrinol* 1999; **13:** 851–65.

39 Danilovich N, Roy I, Sairam RM. Ovarian pathology and high incidence of sex cord tumors in follitropin receptor knockout (FORKO) mice. *Endocrinology* 2001; **142:** 3673–84.

40 Walker GR, Schlesselman JJ, Ness RB. Family history of cancer, oral contraceptive use, and ovarian cancer risk. *Am J Obstet Gynecol* 2002; **186:** 8–14.

41 Schildkraut JM, Moorman PG, Halabi S, Calingaert B, Marks JR, Berchuck A. Analgesic drug use and risk of ovarian cancer. *Epidemiology* 2006; **17:** 104–07.

42 Cramer DW, Harlow BL, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. Over-the-counter analgesics and risk of ovarian cancer. *Lancet* 1998; **351:** 104–07.

43 Reese J, Zhao X, Ma WG, Brown N, Maziasz TJ, Dey SK. Comparative analysis of pharmacologic and/or genetic disruption of cyclooxygenase-1 and cyclooxygenase-2 function in female reproduction in mice. *Endocrinology* 2001; **142:** 3198–206.

44 Downs SM, Longo FJ. An ultrastructural study of preovulatory apical development in mouse ovarian follicles: effects of indomethacin. *Anat Rec* 1983; **205:** 159–68.

45 Downs SM, Longo FJ. Prostaglandins and preovulatory follicular maturation in mice. *J Exp Zool* 1983; **228:** 99–108.

46 Tung KH, Wilkens LR, Wu AH, et al. Effect of anovulation factors on pre- and postmenopausal ovarian cancer risk: revisiting the incessant ovulation hypothesis. *Am J Epidemiol* 2005; **161:** 321–29.

47 Chiaffarino F, Pelucchi C, Parazzini F, Negri E, Franceschi S, La Vecchia C. Reproductive and hormonal factors and ovarian cancer. *Ann Oncol* 2001; **12:** 337–41.